TCR NOTES

* mL → uL!
more than 24 samples → 96 well plate
* can do sample prep night before + put homogenates in -80°C, till next day.
* make sure you have enough of each buffer → calculate for n+10%, so you know.
* spray hood + other surfaces w/ ethanol.

RNA Extraction with RNA SPRI Beads:

sample prep

1. Pull out and place in a tube the amount of RNA beads you need (90uL/sample; n+1 amount) to allow them to acclimate to room temp. 90 • n+10% (take 10% of n and add it)
2. Place each sample in a 2mL tube with 1 clean BB.
3. Add 100uL lysis buffer to each sample. Shake the tubes in the Tissue Lyzer for 1-3min at 30hz.
4. Add 60uL 100% isopropanol to each tube. Mix by pipetting and incubate for 1 minute.
5. Spin samples down in the centrifuge for 1 min at 12,000g. → RCF NOT RPM
6. In a 96-well PCR plate (many samples) OR in autoclaved 1.5mL tubes (few samples), add 90uL RNA magnetic beads to each well/tube and 10uL Lysis/Binding Enhancer (LBE). Mix well. → centrifuge
7. Move the samples from centrifuge tubes to either the plate or tube. Pipette up and down 15-20 times to mix the sample and beads together well. Incubate at room temp for 5-10 minutes. (binds to beads) → try not to get bubbles
8. Place the plate/tubes on a magnetic rack and allow the beads to separate (2-3min). Remove the supernatant. NOTE: The buffers in this step cause the beads to come off the magnet easily, so be gentle when removing supernatant to not remove beads.
9. Pull plate/tubes off the magnetic rack. Add 150uL RNA Beads Wash Buffer (WB) 1, pipette up and down 10-15 times to mix the buffer and beads well. Incubate at room temp for 2 min. → take off rack → no bladder!
10. Place the plate/tubes on the magnetic rack and allow the beads to separate (2-3min). Remove the supernatant being careful not to remove beads. → use multichannel set @ 150uL → yellow tip
11. Pull plate/tubes off the magnetic rack. Add 150uL WB2, pipette up and down 15-20 times to mix the buffer and beads well. Incubate at room temp for 2 min. (n+10% → reservoir (150 • n+10%)
12. Place the plate/tubes on the magnetic rack and allow the beads to separate (2-3min). Remove the supernatant being careful not to remove beads. During the separation of beads and WB2, make a mixture of DNase and DNase buffer for your samples. For each sample add 24uL WB2, 3uL DNase and 3uL DNase Buffer. Make n+1 amount. → n+10% amount (separate?)
13. After removing WB2 supernatant, remove the plate/tubes from the magnet. Add 30uL of the DNase mixture to each sample. Pipette up and down 10-15 times to mix beads and DNase together. Incubate at room temperature for 15-30min.
14. After incubating, add 100uL of the binding buffer to each of the samples. Pipette up and down to mix well. Incubate for 3-5min. (n+10% ? 3?3?)
15. Place the plate/tubes on the magnetic rack and allow the beads to separate (2-3min). Remove the supernatant being careful not to remove beads.
16. Pull plate/tubes off the magnetic rack. Add 150uL WB2, (n+10%?) pipette up and down 15-20 times to mix the buffer and beads well. Incubate at room temp for 2 min. Place the plate/tubes on the magnetic rack and allow the beads to separate (2-3min). Remove the supernatant being careful not to remove beads. Repeat this for a total of 2 times. (steps 15 + 16)
17. On the second removal of WB2, leave the plate/tube on the magnetic rack. Let the beads air dry for 3-5min, but being careful to not over dry them (over drying causes lower yield). (-? → closer to 3) (on magnetic rack)
18. Remove the plate/tubes from the magnetic rack after drying. Add 25-50uL autoclave ddH_2O to each sample to elute the RNA. Pipette up and down 10-15 times to mix the beads and water well. Incubate at room temp for 5 min.
19. Place the plate/tubes on the magnetic rack and allow the beads to separate (2-3min). Remove all but 2uL of the water from the plate/tubes being careful not to remove beads. Place the RNA in new tubes.
20. Qubit the RNA for concentration and store at -80°C for downstream experiments. Take off 23uL

24 or thicker area facing outwards

*Take a photo of order of tubes by marker may rub off in tissue lyzer

*Balance it!

Count of tubes on each side

Take pic of tubes in rack so you know what sample is in each well.

Edsheet

- any time something goes into hood, spray w/ ethanol.
- spray gloves down if you leave hood.
- can set timers on phone - gloves off, use, new gloves

* n+10% only in steps.

100uL x n+10% = needed volume

MOSQUITO